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Trans-dermal fentanyl patches are a cost-effective method of long-term analgesic delivery following corneal exposure to sulfur mustard vapor

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14. ABSTRACT

Analgesic pain management for acute pain resulting from ocular sulfur mustard (SM) exposure in rabbits involves delivery of the opioid receptor agonist buprenorphine using subcutaneously implanted osmotic pumps. The combination of an invasive surgery, risk of post-surgical infection and central effects makes this system suboptimal. Alternatively, fentanyl, a synthetic opioid with predominantly mu receptor agonist activity, is significantly more potent than buprenorphine, with fewer associated CNS side effects. Fentanyl is commercially available as a transdermal patch (Duragesic®), obviating the need for surgery and the risks of secondary complications. To evaluate fentanyl transdermal patches (FPs) as a novel analgesic delivery system for the acute pain associated with ocular SM exposures in rabbits, we studied the analgesic efficacy, conducted resource cost-comparisons and assessed the ease of use and compatibility of FPs with experimental protocols. We found that FP use resulted in decreased symptoms of rabbit pain and distress, reduced experimental duration by 39%, reduced personnel requirements by 14%, and lowered total experimental costs by 43%, or over $8,400 per each 16-rabbit exposure. These data suggest that FPs provide efficient, cost-effective and humane analgesia following ocular SM exposure, while eradicating the discomfort, distress and risk associated with implantation and removal of osmotic pumps.

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Abstract

Analgesic pain management for acute pain resulting from ocular sulfur mustard (SM) exposure in rabbits involves delivery of the opioid receptor agonist buprenorphine using subcutaneously implanted osmotic pumps. Although this system provides effective pain control, the combination of an invasive surgery, risk of post-surgical infection and central effects makes it suboptimal. Alternatively, fentanyl is a synthetic opioid with predominantly mu receptor agonist activity that is significantly more potent than buprenorphine, but with fewer associated CNS side effects. Fentanyl is commercially available as a transdermal patch (brand name Duragesic), obviating the need for surgery and the risks of secondary complications. To evaluate fentanyl transdermal patches (FPs) as a novel analgesic delivery system for the acute pain associated with ocular SM exposures in rabbits, we studied the analgesic efficacy, conducted resource cost-comparisons and assessed the ease of use and compatibility of FPs with experimental protocols. We found that FP use resulted in decreased symptoms of rabbit pain and distress, reduced experimental duration by 39%, reduced personnel requirements by 14%, and lowered total experimental costs by 43%, or over $8,400 per each 16-rabbit exposure. These data suggest that FPs provide efficient, cost-effective and humane analgesia following ocular SM exposure, while eradicating the discomfort, distress and risk associated with implantation and removal of osmotic pumps.

Introduction

Sulfur mustard (2,2'-dichloroethylsulfide; SM) is a highly reactive, bifunctional chemical that alkylates proteins and nucleic acids. Battlefield deployment of SM as a chemical weapon in WWI and the Iran-Iraq war resulted in over 210,000 British and Iranian casualties, 90% of which presented with ocular lesions (Papirmeister et al., 1991). In humans, the acute stage of ocular SM toxicity involves dose-dependent morbidities caused by vesication of the corneal epithelium (CE) and keratocytosis in the epithelium and stroma. Eyes are the most sensitive organ to SM injury, and although mild exposures typically resolve uneventfully, those individuals that receive moderate or severe exposures (exceeding 100 $\mu g \cdot min/m^3$) exhibit three distinct clinical trajectories: (i) injury resolution, after which the victim remains asymptomatic; (ii) persistent keratitis that ultimately results in corneal degeneration (chronic injury); or (iii) an asymptomatic period followed by reemergence of lesions (delayed-onset injury) (Javadi et al., 2007; Papirmeister et al., 1991). The latter two trajectories comprise the phenomenon known as mustard gas keratopathy (MGK), which has been diagnosed in 16% of casualties receiving a moderate or worse exposure (Khateri et al., 2003; Mousavi et al., 2009). The mechanism underlying the development of MGK is unknown and is one focus of our research effort.

Rabbits have been a model system for ocular SM injury for over 60 years (Mann and Pullinger, 1944; Petrali et al., 2000). They exhibit several anatomical and physiological features that facilitate ocular toxicology research, such as a large corneal to sclera ratio, a relative insensitivity to corneal drying and a low frequency of spontaneous epithelial lesions (Marzulli and Maibach, 1996). In addition, rabbit and human corneas exhibit significant structural similarities, and although rabbits are four-fold less sensitive to ocular SM injury than humans, at normalized doses they display similar sequelae and injury progression (Gates and Moore, 1946; Mann and Pullinger, 1944). Recently, rabbit exposure models have been used to evaluate candidate treatments, test novel ocular delivery systems and evaluate long-term SM toxicology in the limbus (Amir et al., 2000; Babin et al., 2004; Gordon et al., 2010; Kadar et al., 2009).

Standard methods for analgesic delivery in New Zealand white rabbits include both injection and implanted osmotic pumps. While the former is minimally invasive, the short window of efficacy requires multiple injections, significantly increasing animal handling, and results in a cyclical dosing pattern. Osmotic pumps enable continuous dosing over prolonged periods (6 d for our purposes) and minimize fluctuations in analgesic control. However, the surgical implantation of osmotic pumps is resource-intensive, requires multiple trained technicians and increases the risks of post-operative irritation and infection. The time required to prepare and implant osmotic pumps introduces significant delays that have to be adjusted for during exposures, and the extent of analgesic relief has to be increased to accommodate the discomfort incurred by the pump itself.

Fentanyl transdermal patches (FPs) offer a novel alternative for analgesic delivery following ocular sulfur mustard exposure. Fentanyl is a potent synthetic opioid of the 4-anilinophenylpiperidine class, with predominantly mu receptor agonist activity. It is reported to be 75 times more potent than morphine in humans (Calis et al., 1992; Yelnosky and Gardocki, 1963). Fentanyl is lipid and water soluble, and is rapidly eliminated from the plasma in humans (Hammargren and Henderson, 1988). Buprenorphine is another opioid agent that is often used as the first-choice analgesic in laboratory animal medicine. Buprenorphine is a partial mu and kappa agonist that induces analgesia along with CNS side effects (Flecknell, 1984). However, the maximal analgesic effect of buprenorphine is significantly less than that of fentanyl, and therefore Buprenorphine is less useful for controlling acute and severe pain (Gades et al., 2000).

Given these advantages, we hypothesized that FPs would offer a less-invasive, more facile and less expensive analgesic delivery system than buprenorphine administration by osmotic pumps while still providing effective pain management. To evaluate this, we determined the comparative resource costs of FPs and osmotic pumps and determined the ability of FPs to provide pain management by screening for signs of rabbit distress following ocular SM vapor exposure.

Materials and Methods

Animals: Thirty-two female New Zealand white rabbits (Charles River Laboratories) weighing 2.5 kilograms each were randomly divided into two equal cohorts and housed individually. Rabbits were provided a controlled diet and watered *ad libitum*. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

Application of analgesic delivery systems:

Fentanyl patch application: One day prior to exposure, unanesthetized rabbits were removed from their cages and placed sternally on a table. Using electric clippers and size forty clipper blades (Oster), a 4-x-4-in patch of hair was clipped dorsally and anterior to the scapula, leaving 0.25 to 0.5 mm of stubble (Fig. 1). Clipping was chosen over shaving as complete depilation causes accelerated absorbance of fentanyl into the bloodstream (Foley et al., 2001). Once the area was clipped, a 25 ug/hr FP was placed on the clipped skin of the rabbit (Fig. 1D). Rabbits were returned to cages and monitored throughout the day.

Buprenorphine pump implantation: One day prior to exposure, unsedated rabbits were removed from their cages and placed sternally on a table. Using electric clippers and size forty clipper blades (Oster), a 4–x-4-in area between the scapulae was shaved, and residual hair was removed with a triple-blade razor. On the day of exposure, rabbits were anesthetized with an intramuscular injection of ketamine (15 mg/kg) and xylazine (7 mg/kg) and primed with a subcutaneous injection of buprenorphine (0.05 mg/kg). Once rabbits achieved a surgical plane of anesthesia, a 1- to 1.5-in cutaneous incision was made between the scapulae, and a pocket was formed by gently separating the skin and fascia with a hemostat. An osmotic pump (Alzet, 2ML1, 10.0 ul/h) designed to administer buprenorphine (0.3 mg/mL) for six days was inserted into the pocket, and the incision was closed with surgical staples. The animal was then transported to the exposure room.

Exposures: On the day of exposure, FP cohort rabbits were anesthetized with an intramuscular administration of 15 mg/kg of ketamine and 7 mg/kg of xylazine. Rabbits in the osmotic pump cohort were prepared as stated above. Rabbit corneas were exposed to SM vapor for 2.5 min using a previously described vapor cup delivery system (McNutt et al., 2011; Milhorn et al., 2010). Two min after exposure, exposed eyes were flushed with 10 mL of sterile saline to remove any residual agent. Upon becoming sternal, food and water were provided *ad libitum*.

Evaluations of Pain and Distress: Animals were monitored for six days following exposure for signs of pain or distress using the metrics described in Appendix A and Table 1.

Fentanyl patch removal: Serial application of FPs was used to ensure rabbits received pain management through 6 d after SM exposure. At this time, the corneal epithelium has regenerated, and the acute lesion has been repaired, reducing the need for acute pain management (McNutt et al., 2011). Since FPs provide persistent analgesic relief for approximately 72 h, they were replaced after 3 d with a fresh patch in the same location. The second patch was removed 4 d later.

Controlled substance policy: As controlled substances, FPs require receipt-to-disposal oversight. However, unlike injectable drugs, FPs are physically accessible while on the rabbits and residual fentanyl on the patches might be misused following their removal. Thus, in coordination with the USAMRICD controlled substance officer, we developed a new substance control policy that provided oversight of each stage of patch use. FPs were tracked using a DA 3949 form at the following points: (1) receipt, when patches were transferred to user control and stored until use; (2) application, when patches were transferred to rabbits; and (3) destruction, when used patches were folded in half and discarded in a "sharps" container. Destruction was witnessed by a second party. Inventories were maintained in a controlled substance log book and audited on a monthly basis or upon request.

Osmotic pump removal: Seven days after pump implantation, rabbits were anesthetized with an intramuscular injection of ketamine (15 mg/kg) and xylazine (7 mg/kg). Surgical staples were removed, the incision was reopened and expired pumps were extracted. The incision was re-stapled, and animals were observed until recovery. Staples were removed 7 d later without anesthesia.

Determination of cost benefits: The average team cost per minute was calculated from the average salary or wage of team members involved in exposures. This calculation only incorporated direct costs and did not include fringe benefits or personnel overhead.

RESULTS:

Application of the FPs: Adhesive FPs were placed on a clipped patch on the dorsal region of the neck, anterior to the scapulae (Fig 1). Upon initial application, most rabbits were unable to dislodge the patch. In one instance where the patch was placed slightly posterior to the scapulae, the rabbit was able to remove the patch with a rear foot. Replacement of the patch higher along the neck prevented further problems. After 72 h, the original FPs were removed and replaced with new FPs in the same location for an additional 96 h. The skin beneath and surrounding the patch did not exhibit any apparent sign of dermal irritation, and the adhesive was sufficient to keep the patch in place for the duration of the experiment. Since there was no surgery associated with FP administration, the only risk of infection was at injection sites, whereas the implantation site had to be monitored for post-surgical infection for 14 days.

Behavioral and physiological metrics of exposed rabbits: Although all rabbits exhibited physiological symptoms consistent with ocular lesions (mild photophobia and tearing) by 1 d after exposure, there were

no significant symptoms of ocular pain or distress with either the FP or the osmotic pump over six days following exposure (measured using behavioral metrics described in Appendix A and Table 1) (McNutt et al., 2011; Milhorn et al., 2010). The most recognizable signatures of distress (shielding or protecting the eyes and aggressive behavior) were not observed in either cohort. The FP-treated animals were more alert and less lethargic than the osmotic pump animals (Figure 2), suggesting that FPs provided effective pain control without incurring physiobehavioral side effects.

Resource cost comparisons between osmotic pumps and FPs: In comparison to the use of osmotic pumps, FPs dramatically reduced time, labor and resource costs. First, the cost per patch was significantly lower than the costs of purchasing and loading an osmotic pump with buprenorphine. Pump implantation took approximately 10 min per animal, whereas FP application took 0.5-1 min. Since there were no surgical procedures involved for the FP cohort, we were able to simultaneously anesthetize multiple animals and expose them as they entered a surgical plane of anesthesia, allowing a more flexible and facile experimental execution. This reduced the total time required to conduct 16 ocular exposures from 4.5 h to 2.5 h and the number of required personnel from 7 to 6. The use of FPs obviated the need for a surgical suite for pump implantation, as well as the need for pump extraction and staple removal. This represents a net decrease in total cost (supplies plus labor) of 43% per experiment. *In toto*, switching to the FP-based analgesic delivery method resulted in significantly reduced reagents costs (Table 2) and labor costs (direct salary costs, not including fringe benefits and indirect costs; Table 3) by a combined total of $525.93 per rabbit.

DISCUSSION:

The purpose of this study was to determine whether percutaneous fentanyl patches were suitable delivery systems for prolonged pain management following corneal SM vapor exposure. In rabbits, FPs produced relatively consistent dosing for 72 h, with a mild erythema at the site of delivery being the only reported complication (Foley et al., 2001). Unlike implanted pumps, FPs do not require invasive surgical procedures, post-surgical surveillance and secondary surgical protocols, decreasing both animal handling and required resources.

The use of FPs resulted in fewer symptoms of distress, required no invasive procedures and involved less handling. Unlike implanted osmotic pumps, FPs incurred no apparent risk of secondary infection since FP administration is non-surgical, simply requiring the application of a self-adhering patch to a clipped dorsal surface. Furthermore, unlike extraction of the exhausted osmotic pumps, removal or replacement of FPs did not require anesthesia.

Rabbits provided with transdermal FP did not exhibit increased symptoms of ocular discomfort and presented with fewer symptoms of general distress than did the buprenorphine cohort. The elevated behavioral changes exhibited by the rabbits with implanted pumps were likely a consequence of the CNS effects caused by the need to apply additional analgesic to (a) compensate for the lower specific efficacy of buprenorphine and (b) mitigate the additional discomfort caused by pump implantation. No differences in ocular outcome were observed between the two cohorts, nor was there evidence of respiratory depression at any time, suggesting that both analgesic systems were sufficiently effective to handle the acute pain resulting from ocular SM exposure and did not influence ocular injury progression or healing.

The majority of cost savings was realized through the streamlining of the exposure protocol, which resulted in decreased animal handling and personnel time. By eradicating surgical pump implantation, the number of technicians was reduced by 14% and the exposure duration decreased by 39%. Additional cost savings were observed by eliminating ancillary procedures such as the removal of osmotic pumps and staples. *In toto*, these changes contributed to a net cost savings of $525.93 per rabbit, for a total savings of over $8,400 per experiment. In a typical year where we perform six 16-rabbit exposures, the total savings is approximately $50,000. If we were to incorporate fringe benefits and other indirect costs, including VMSB personnel time, the actual savings would be significantly higher.

Overall, these data suggest that FPs provide efficient, cost-effective and humane analgesic delivery following corneal SM exposure while eradicating the discomfort, distress and risk associated with implantation and removal of osmotic pumps.

Figure 1. Photographic images of preparation and application of the fentanyl transdermal patch. (A) The upper back/lower neck regions of rabbits are clipped in preparation of patch application. (B, C) Patch packaging and the patch itself. (D) Application to the shaved surface of the rabbit.

Figure 2. Pain and distress scores were calculated from behavioral characteristics (measured according to Table 2) and compared between cohorts that received a fentanyl transdermal patch versus osmotic pump.

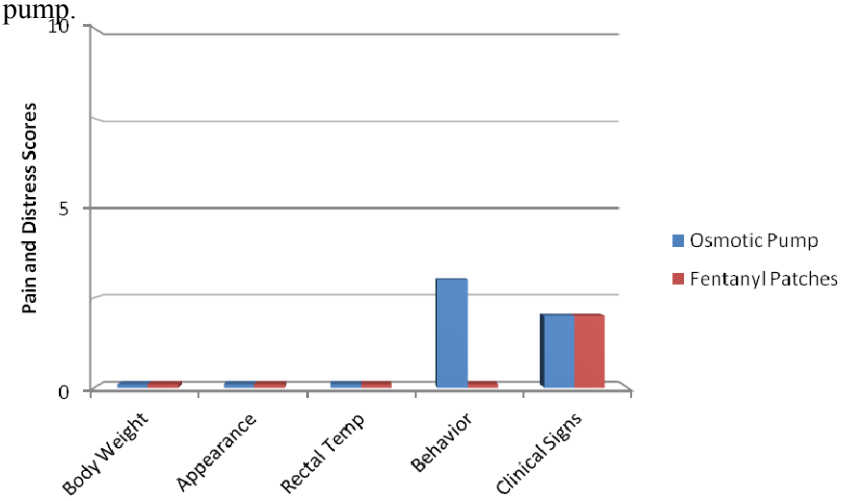

Table 1: Scoring of Pain or Distress

	Pain Scores				
Pain Score Categories	**0**	**2**	**3**	**5**	**10**
Body Weight	< 5 % decrease	6-10 % decrease	-	11-25% decrease	> 25% decrease
Appearance	Normal	Huddled and mild piloerection	Huddled and moderate piloerection	Huddled, ungroomed, and severe piloerection	
Rectal Temp. (99 - 103.1 F)	Normal	± 0.5 F of normal range	-	± 1 F of normal range	± 2 F of normal range
Behavior	Normal	Responsive when stimulated, decreased appetite	Mildly lethargic and responsive	Lethargic and Mildly unresponsive, decreased appetite and water intake	Unresponsive or moribund
Clinical Signs	Normal	ocular discharge	Mild respiratory distress	Moderate respiratory distress, ataxia and/or dehydration	Severe respiratory distress and/or severe dehydration.

Each pain scale has a corresponding action plan.

Score

0 – 4 total score or <1 score in a category: No intervention

4 – 9 total score or >1 score in a category: Increase the frequency of observation and/or consider euthanasia.

>=10: Euthanize animal

Table 2: Comparison of the supply costs per rabbit associated with using fentanyl patches and buprenorphine-loaded osmotic pumps.

Osmotic pumps					FPs			
Analgesic application	**Cost**	**QTY**	**Total Cost**		**Analgesic application**	**Cost**	**QTY**	**Total Cost**
Osmotic pump	$ 25.10	1	$ 25.10		FP	$ 6.58	2	$ 13.16
Rabbit	$ 70.95	1	$ 70.95		Rabbit	$ 70.95	1	$ 70.95
Ketamine half dose	$ 0.55	2	$ 1.10		Ketamine half dose	$ 0.28	1	$ 0.28
Rompun	$ 0.42	2	$ 0.84		Rompun	$ 0.42	1	$ 0.42
Buprenex pre-surgical loading	$ 5.16	2.5	$ 12.90		Buprenex	$ 0.00	0	$ 0.00
Staples	$ 0.52	8	$ 4.16		Staples	$ 0.00	0	$ 0.00
Osmotic pump accessories					**FP accessories**			
Scalpel blades	$ 0.64	2	$ 1.28		Lab coats	$ 5.78	11	$ 63.58
Nolvasan	$ 0.07	1	$ 0.07		Nitrile gloves	$ 0.16	2	$ 0.32
Surgical drapes	$ 7.93	1	$ 7.93		Facemasks	$ 0.43	11	$ 4.73
Gauze	$ 0.28	2	$ 0.56		Clipper blades	$ 37.63	1	$ 37.63
Surgical gowns	$ 7.73	1	$ 7.73		Shaving razors	$ 2.15	1	$ 2.15
Surgical gloves	$ 12.14	2	$ 24.28		Syringes/needles	$ 1.56	2	$ 3.12
Face masks	$ 0.43	16	$ 6.88					
Clipper blades	$ 37.63	1	$ 37.63					
Shaving razors	$ 2.15	1	$ 2.15					
Syringes/needles	$ 1.56	4	$ 6.24					
Lab coats	$ 5.78	10	$ 57.80					
		Total Cost:	**$ 267.60**				**Total Cost:**	**$ 196.34**
Total reagent savings per rabbit: $71.26								

Table 3: Comparison of the direct labor costs per rabbit associated with using fentanyl patches and buprenorphine-loaded osmotic pumps. Labor costs are based on $0.51 per person·minute, which is the average wage of our research team. This calculation incorporated direct costs only, and does not include fringe benefits or personnel overhead. The number and cost of personnel involved in animal procedures are based on USAMRICD requirements, and may vary based on regulatory context. These values also do not include ancillary support services, such as veterinary support, which would be involved predominantly with osmotic pump implantations. Given these two approximations, the calculated savings are likely an underestimate of the true savings.

Osmotic pumps	**Time (min)**	**# Personnel**	**Direct cost**		**Fentanyl patches**	**Time (min)**	**# Personnel**	**Direct cost**
Labor Cost per Rabbit	aver $0.51/min				**Labor Cost Per Rabbit**	aver $0.51/min		
Osmotic pump loading	7.5	1	$3.83		Rabbit prep and FP application	4	2	$4.08
Surgical prep	9.5	2	$9.69		Surgery	0	0	$0.00
Surgery	15	2	$15.30		Experiment duration	150	6	$459.00
Exposure duration	245	7	$874.65		Post exposure recovery time	45	1	$22.95
Post-exposure recovery time	45	1	$22.95		FP removal/replacement	4	2	$4.08
Pump extraction/staple removal	18	2	$18.36					
		Total Cost:	**$ 944.78**				**Total Cost**	**$ 490.11**
Total personnel savings per rabbit: $454.67								

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Appendix A: Markers of Pain or Distress in the Laboratory Rabbit

Purpose: To assess welfare and pain management in rabbits during periodic observations and manipulations by research staff.

Scope: Research technicians will evaluate rabbit behavior prior to and during research manipulations and periodic observations. Findings that meet the following criteria and/or suggest animal distress will be reported immediately to the clinical veterinary staff. Decisions will then be made regarding treatment or early endpoint selection based on animal welfare concerns or preserving research integrity.

1. **Agitation:** Agitation in rabbits is uncommon and is a significant indicator of discomfort, annoyance, fear or pain. Animals will be observed for the following markers of agitation:
    - Frequent/constant thumping of hind feet
    - Repetitive circling of cage
    - Vocalization when not being handled or excessive growling during handling
    - Protection of exposed eyes

2. **Irregular Movement:** Rabbits normally do not display any signs of discomfort or weight shifting when they do not have some sort of ambulatory impairment. They will typically walk on all fours and will either walk normally, or they will lie calmly in their cages when undisturbed. The ocular research should not result in ambulatory disorders, except in instances of nerve fiber aggravation following injections. Key indicators that an animal may be experiencing ambulatory pain and or discomfort are as follows.
    - Frequent position changes
    - Limping
    - Appearance of not bearing weight on any limb or inability to use any limb

3. **Appetite loss:** Rabbits continually nibble at food and readily consume fresh fruits and vegetables, although like most animals they exhibit individual preferences. Evidence of a lack of appetite for food and enrichment items will be noted and reported.

4. **Abnormal Excreta (veterinary technician):** Normal fecal output in rabbits appears as round firm pellets. Normal urine output in rabbits is yellow and either viscous or clear. Abnormal findings will include the following.
    - Lack of excreta (urine or feces)
    - Bloody excreta (urine or feces)
    - Loose, watery or mucosal stool

5. **Lack of Grooming or Excessive Grooming:** Rabbits are fastidious by nature and the following signs of poor grooming are often a signature of distress:
    - Excessively scruffy coat

- Coat stained excessively with feces and/or urine
- Matting of fur

6. **Abnormal Eye Issues:** Animals utilized under this protocol may exhibit corneal edema, hazing, ulceration, lacrimation, photophobia, discharge or other exposure-related symptoms. Abnormal findings in exposed eyes include unexpected sequelae or expected sequelae of such severity that the ocular integrity is jeopardized. Unusual observations in non-exposed eyes will be noted and reported. While closing of the eyelid is normal due to photophobia, attempts to protect the eye will be noted and reported.

7. **Lacerations and Abrasions:** All lacerations, wounds or abrasions anywhere on the rabbit will be reported immediately, regardless of whether the animal is exhibiting signs of discomfort. The presence of blood (not associated with blood draws) will be noted and reported immediately.